There was also a strong correlation between Eh GSH/GSSG and CIMT (r = 0.60, p- < 0.01), and an inverse correlation between GSH and CIMT (r = - 0.52, p- < 0.01). We then stratified subjects based on their CIMT into those with CIMT > 0.6 mm and those with CIMT ≤ 0.6mm. Differences in the various markers between the 2 groups are shown below.

Variable (Units)	CIMT ≤0.60 mm	CIMT > 0.60 mm	P-Value
FMD (%)	7.68% (+/-2.4)	5.59% (+/- 3.1)	0.02
Eh GSH/GSSG (mV)	-126.2 (+/- 6.3)	-118.8 (+/- 7.2)	0.01
GSH (uM)	2.51(+/-0.86)	1.84 (+/- 0.69)	0.03
GSSG (uM)	0.24 (+/-0.11)	0.23 (+/- 0.12)	NS

After multivariate analysis, both FMD and Eh GSH/GSSG were independent predictors of CIMT. **Conclusion:** These findings suggest that markers of oxidative stress such as Eh GSH/GSSG may be helpful in predicting individuals at risk for early atherosclerosis. This predictive value appears to be independent of endothelial function.

POSTER SESSION

1177 Drug Therapy and Endothelial Function

Tuesday, April 01, 2003, Noon-2:00 p.m.
McCormick Place, Hall A
Presentation Hour: 1:00 p.m.-2:00 p.m.

1177-126 Additive Effects of Angiotensin Converting Enzyme Inhibitor Combined With Statin on Inflammation and Fibrinolysis in Hypercholesterolemic Patients With Coronary Artery Disease

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Background: Because the mechanisms of the biological effects of statins and antiotensin converting enzyme inhibitor therapies differ, we studied the vascular responses to these therapies in hypercholesterolemic patients with coronary artery disease (CAD). **Methods**: We administered simvastatin 20 mg and placebo or ramipril 10 mg daily during 2 months with washout 2 months to 30 hypercholesterolemic patients with CAD. This study was randomized, double-blind, placebo-controlled, crossover in design. *=P<0.05;**=P<0.01;***=P<0.001 vs. Baseline. Data= mean+SD.
Results: Simvastatin alone or combined with ramipril significantly changed lipoproteins, and improved the percent flow-mediated dilator response (FMD) to hyperemia from 4.57±1.63 to 5.96±1.88 by 38±40% and from 4.49±1.39 to 6.28±1.76 by 53±77%, respectively (both P<0.001) and reduced plasma levels of nitrate from 97±47 to 90±52 uM by 4±58% and from 97±47 to 79±44 uM by 10±47%, respectively (P=0.413 and P=0.037, respectively), and plasma levels of malondialdehyde (MDA), a marker of free radical from 1.41±0.66 to 1.11±0.60 uM by 13±44% and from 1.40±0.79 to 0.97±0.57 uM by 20±48%, respectively (P=0.005 and P<0.001, respectively), and MCP-1 by 7±24% and by 13±18%, respectively (P=0.010 and P<0.001, respectively), and C-reactive protein by -7±107% and by 15±45%, respectively (P=0.073 and P=0.009, respectively) , and PAI-1 antigen by -9±52% and by 11±38%, respectively (P=0.971 and P=0.014, respectively). However, simvastatin combined with ramipril changed to greater extent FMD, and plasma levels of nitrate, MDA, MCP-1, CRP, and PAI-1 antigen than simvastatin alone.
Conclusions: Compared with simvastatin alone, added ramipril to simvastatin showed additive effects on flow-mediated dilation and the plasma levels of nitrate and MDA, inflammation markers and fibrinolysis potential markers in hypercholesterolemic patients with CAD.

1177-127 In Vivo Evidence for Adriamycin-Induced Uncoupling of Endothelial Nitric Oxide Synthase

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Background: Adriamycin (ADR) is a commonly used anti-neoplastic agent that is well known to induce cardiotoxicity. While the mechanisms of this effect are not entirely clear, evidence suggests that it is a free radical mediated process. We hypothesized that administration of a single dose of ADR is associated with abnormalities in nitric oxide (•NO) availability and endothelial nitric oxide synthase related superoxide ($O_2^{\bullet-}$) generation.
Methods and results: A single dose of ADR (10 mg/kg IV) in rabbits resulted in reduced peak relaxations to the agonists acetylcholine (Ach, 99±2% to 57±4%) and A23187 (92±3% to 57±5%) and a shift in the ED_{50} values. Peak relaxations to NTG were also altered (104±1 to 80±6) with no change in ED_{50} concentration. Exposure of aortic segments to ADR for 30 minutes resulted in dose dependent $O_2^{\bullet-}$ generation, as measured by electron spin resonance (ESR) that was abolished by endothelial denudation and incubation with diphenylene-iodinuim (10 μM) but not L-NMMA (10μM). Experiments with recombinant eNOS protein, confirmed a dose dependent decrease in •NO generation as measured by L-Arginine-Citrulline assays and increase in $O_2^{\bullet-}$ production with ADR. Brachial artery flow mediated dilation (FMD) in patients undergoing ADR administration (60 mg/m^2) was markedly attenuated after a single dose (6.5±1.0 to 2.5±1.1%, p=0.0004, time to end of infusion 27±8 minutes). Serum nitrite and nitrate concentrations fell from 50+6 μmol/L pre-ADR to 33+6 μmol/L post ADR infusion (p=0.0005) while serum concentrations of CD/131 thrombomodulin and vWF activity remained unchanged after ADR infusion (36±13 to 52±22% ng/ml vs. 3.25±0.98 to 3.01±0.91% (p=NS for pre vs. post for both).
Conclusions: ADR administration results in rapid depletion of •NO levels as evidenced by attenuation of agonist dependent responses in rabbits and reactive hyperemic responses in human brachial arteries associated with reductions in serum •NO levels. ESR measurements in aortic segments strongly suggest an eNOS origin for free radical production. These findings may have implications for cardiovascular complications noted with ADR.

1177-128 Lipophilic Statin-Induced Antiangiogenesis Requires the Inhibition of Ras Farnesylation in Human Coronary Artery Endothelial Cells

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Background: While a recent report suggested that inhibition of Ras might be an effective anti-angiogenic therapy, it is unclear whether Ras mediates 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor (statin)-induced signal transduction. Therefore, we investigated that Ras plays a role in statin-mediated promotion of angiogenesis in human coronary artery endothelial cells (HCECs).
Methods: We developed an in vitro model of HCECs tube formation on a matrix gel. pEGFP(enhanced green fluorescent protein)-wild type (WT) Ras and pEGFP-dominant negative (N17) Ras expression vectors were constructed. Untransfected or transfected HCECs were seeded on a matrix gel and grown in medium supplemented with 5 % serum and without endothelial cell growth supplement for 24 hours. In some experiments, cells were cultured in the presence or absence of different kinds of reagents.
Results: Lipophilic statins (simvastatin, atrovastatin, itavastatin and cerivastatin), but not a hydrophilic statin (pravastatin), inhibited serum-induced endothelial tube formation. Inhibition of p42/44 MAPK (mitogen-activated protein kinase) activity, but not the inhibition of p38 MAPK activity, also suppressed serum-induced tube formation. The addition of mevalonic acid rescued simvastatin-induced inhibition. Farnesylpyrophosphate, which translocates Ras from the cytoplasm to the cell membrane, also blocked this inhibition. In addition, farnesyltransferase I inhibitor, which inhibits Ras farnesylation, blocked tube formation. RasN17 also inhibited serum-induced tube formation. Moreover, simvastatin blocked epidermal growth factor-induced EGFP-RasWT translocation.
Conclusions: Our results identified Ras as a key player in the anti-angiogenic action and signaling pathway of statins. This is the first comprehensive analysis of the role of Ras in the statin-induced modulation of an angiogenic effect. The inhibition of angiogenesis by statin could explain, at least in part, the protective effect of these drugs against atherothrombotic events, which was greater than expected simply based on the cholesterol decrease.

1177-129 Angiotensin II Type-1 Receptor Antagonist Inhibits Vascular Phenotypic Change and Remodeling in Intramyocardial Arteries by Reducing Oxidative Stress Through Upregulation of Superoxide Dismutase

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Background: Angiotensin II type-1 receptor antagonists reduce reactive oxygen species (ROS) generated by activated NAD(P)H oxidase and vascular remodeling in hypertension, however, the effect of ARAs on the scavenging enzymes related to ROS is unclear. We have examined whether Angiotensin II type-1 receptor antagonists may inhibit vascular remodeling and smooth muscle (SM) cell phenotypic change in intramyocardial arteries via ROS-scavenging enzymes.
Methods: Male stroke-prone SHR (SHRSP) were randomized and treated for 6 weeks with vehicle, AIIA (E4177; 30 mg/kg/day), or angiotensin-converting enzyme (ACE) inhibitor (cilazapril; 10 mg/kg/day).
Results: Within 6 weeks of treatment, both drugs showed equipotent effects on blood pressure, left ventricular hypertrophy and fibrosis, synthetic-type myosin heavy chain (MHC) NMHC-B/SMemb, and endothelial NO synthase expression in the heart. Furthermore, compared with the vehicle SHRSP group, E4177 showed a greater reduction of the wall-to-lumen ratio in intramyocardial arteries by reducing ROS assessed with isoprostanes and thiobarbituric acid reactive substances by inhibiting NAD(P)H oxidase essential subunit p22phox and upregulating Cu/Zn superoxide dismutase expression, and also inhibiting phenotypic modulation by reducing synthetic-type MHC NMHC-A and upregulating contractile-type SM-MHC SM2 in the heart more effectively than did cilazapril.
Conclusions: Angiotensin II type-1 receptor antagonists may inhibit vascular remodeling and phenotypic change in the heart of SHRSP by reducing ROS via modulation of the expression of not only the generating enzymes but also the scavenging enzymes related to ROS more efficiently than ACE inhibitor, and strategies aimed at upregulating the scavenging enzymes in addition to downregulating the generating enzymes related to ROS may have therapeutic potential against vascular remodeling in hypertension.

1177-130 Endothelial Function and Slow Coronary Flow

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Objectives- The aim of the study was to determine endothelial function in patients with SCF by using flow-mediated dilatation tecnique.
Background- Slow coronary flow (SCF) in normal coronary angiogram is a well-recognized clinical entity, but its etiopathogenesis remains unclear.
Methods - Coronary flow was quantified using the corrected Thrombosis In Myocardial

Infarction (TIMI) frame count (CTFC) method. Endothelial function was studied in 27 patients with SCF (23 male, 4 female, mean age 47.6± 8.7) and in 30 subjects (22 male, 8 female, mean age 47.5±7.4) with normal coronary flow (NCF).
Results- The mean CTFC ($CTFC_m$) was significantly higher in patients with SCF (45.6±9.6 frames, p<0.001). Reactive hyperemia was significantly lower than that in the NCF group (189±87 % vs. 310±176%, p<0.01). The flow-mediated diameter increase in SCF group was significantly smaller than that in the NCF group (3.48±0.10 % versus 9.11±0.10 %, p<0.001). The percent nitroglycerine-induced (NTG-induced) dilatation was not signifacantly different between patients with SCF and subjects with NCF (16.8±1.1 % versus 17.1±1.1 %, p=0.87). Simple regression analysis showed that $CTFC_m$ was strongly and inversely related to percent of FMD (r=-0.29, p<0.01) in all subjects. When the patients with SCF were excluded, $CTFC_m$ was still inversely related to percent FMD (r=-0.36, p<0.05).The $CTFC_m$ was also inversely related to NTG-induced dilatation in the 57 subjects (r=-0.23, p<0.05). Multiple regression analysis showed that the $CTFC_m$ was inversely related to percent of FMD only (r=-0.37, p<0.05). ***Conclusions-*** These findings suggest that endothelial function is impaired in subjects with SCF and the CTFC was well correlated with endothelial dysfunction.

1177-131 Women With Cardiac Syndrome X Exhibit Features of the Insulin Resistance Syndrome and Impaired Peripheral Microvascular Function

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Background. Anginal chest pain despite angiographically normal coronary arteries in the context of a positive exercise test is a common problem. A proportion of this group, labelled as cardiac 'syndrome x' (CSX), have evidence of myocardial ischaemia. We sought to characterise women with CSX in terms of their metabolic parameters and peripheral microvascular function (MF) as compared with controls
Methods. We recruited 56 women fulfilling the criteria for CSX along with 25 healthy matched controls (HC group). Fasting blood samples and clinical measurements were obtained. Forearm cutaneous microvascular function was assessed by iontophoresis of topically applied acetyl choline and sodium nitroprusside and laser Doppler imaging
Results. There were significant differences between the CSX and HC with regards to indices of insulin resistance (p<0.001), systolic blood pressure (p<0.012), triglycerides (p<0.01), HDL-cholesterol (p=0.012), C-reactive protein (p<0.005), body mass index (BMI) (p<0.001) and serum leptin (p<0.001). Peripheral MF, both endothelium-dependent and independent, was impaired in the CSX group compared to the HC group (p<0.001) and circulating vascular markers (von willebrand factor, tissue plasminogen activator, and cellular adhesion molecules) were also different between the 2 groups (p<0.001). Adjusting for BMI attenuated the differences in the metabolic parameters as did adjustment for insulin. However the differences were further attenuated after correction for serum leptin. The differences in MF remained highly significant even after adjustment
Discussion. Subjects with CSX exhibited features of the insulin resistance syndrome. Attenuation of these differences after correction for leptin highlights the importance of adiposity and insulin resistance in CSX. Impairment of both endothelial-dependent and independent MF suggests not only endothelial dysfunction but more generalised vascular smooth muscle dysfunction which may be important in the aetiology of myocardial ischaemia. Adjusting for leptin and insulin did little to attenuate these differences in MF suggesting other factors play a critical role

1177-132 Spironolactone Improves Endothelial Function in Congestive Heart Failure Patients on Angiotensin Converting Enzyme Inhibitors

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Background: Despite angiotensin converting enzyme inhibition (ACEI), endothelial dysfunction persists in patients with congestive heart failure (CHF). Aldosterone inhibition may ameliorate this dysfunction. We studied the effects of spironolactone on endothelial function in stable CHF patients on optimal doses of ACEI.
Methods: Twenty patients on optimal CHF therapy (age 64 ± 11 years; ejection fraction 24 ± 9%) were treated with spironolactone (25 mg po daily). Brachial artery diameter was measured with ultrasound before and after hyperemia - defined as flow-mediated dilation(FMD), and sublingual nitroglycerin (NTG) at baseline, 4, and 8 weeks. The percent increase in brachial artery diameter with FMD or NTG was defined as : [Final diameter - Baseline diameter]/Baseline diameter x 100(%).
Results: Basal blood pressure, heart rate, and body weight did not change over the study period. Interobserver variability in FMD measurement showed a mean difference of 0.5 ± 0.5% (r=0.97). Compared to baseline, FMD improved significantly at 4 weeks (p=0.017) and persisted at 8 weeks (p=0.08). NTG dilation was unchanged over time. Changes in FMD were not related to age, gender, ejection fraction, presence of diabetes or statin use.
Conclusion: Spironolactone improves endothelial function as early as 4 weeks in CHF patients on ACEI. This effect may be due to reversal of aldosterone impairment of endothelial nitric oxide activity, and may contribute to its clinical benefit in CHF.

TABLE: EFFECTS OF SPIRONOLACTONE ON BRACHIAL ARTERY REACTIVITY

	INITIAL VISIT	4 WEEKS	8 WEEKS
SYSTOLIC BP (mmHg)	125 ± 19	117 ± 11	122 ± 14
HEART RATE (beats/min)	69 ± 11	70 ± 9	65 ± 8
BODY WEIGHT (kg)	82 ± 15	88 ± 12	85 ± 11
FMD (%)	5.5 ± 4.3	9.3 ± 5.4*	9.0 ± 4.9**
NTG-DILATION (%)	16.2 ± 9.6	17.8 ± 6.0	14.4 ± 7.1

*p=0.017; **p=0.08

1177-133 Endothelial Function of Coronary Arteries Distal to a Radioactive Stent

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Background: Coronary artery endothelial dysfunction distal to implanted stents in angioplasty patients has been shown to persist for 6 mo; this may be due in part to the local tissue reactions to stent implant with release of paracrine factors to the downstream vasculature. Radioactive stents alter the local tissue reaction including inhibition of cellular proliferation and potentiation of local inflammation. To evaluate the effect of radioactive stent implant on distal vessel function, we studied vasomotor reactivity of coronary vessels downstream of stent implant. ***Methods:*** Segments of pig coronary arteries 2-cm distal to radioactive Au-198 stents (RAD; n=12, 20 μCi) and non-radioactive Au-197 stents (sham; n=9) were studied one month after stent implant. Endothelium-dependent and –independent functions were investigated in an organ-chamber apparatus. ***Results:*** Contractile responses to KCl and $PGF_{2\alpha}$ were similar in both groups as well as to normal reference vessels. After incubation with L-NAME, contraction to $PGF_{2\alpha}$ was significantly increased in RAD (2.11±0.40g vs. 3.71±0.40g; P<0.01) but not in sham (1.86±0.40g vs. 2.75±0.42g; NS). Dose-responses to endothelium-dependent, receptor-independent (A23187) and endothelium-independent (sodium nitroprusside) relaxations were similar in the two groups. However, maximal dose relaxation (0.1nM) to substance P in RAD showed a strong trend to be higher than in non-radioactive group (47.8±7.9% vs. 20.2±11.1%; P=0.063) in relation to normal reference segments (50.2±11.5%; results not shown). This difference was completely abolished in the presence of NO synthase blockade using L-NAME. ***Conclusions:*** Endothelium-dependent receptor-mediated coronary vasomotor sensitivity but not endothelium-dependent non-receptor-mediated sensitivity was augmented by the presence of a radioactive stent just upstream. The presence of a radioactive stent upstream also increased contractile response to $PGF_{2\alpha}$ when NO synthase activity was blocked. These findings suggest that radioactive stents alter the local tissue environment in a manner that chronically influences the contractile and relaxant function of the downstream coronary vasculature.

1177-134 Effects of Metoprolol on Oxidant Stress Parameters and Nitric Oxide Bioavailability in Angiotensin II Induced Hypertension

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Arterial hypertension and coronary artery disease are associated with activation of the renin-angiotensin-system. For both diseases ß-receptor-inhibitors are part of standard therapy. Angiotensin II (AII) induces endothelial dysfunction and formation of reactive oxygen species. We sought to determine the effects of metoprolol on AII-induced oxidant stress and the NO-pathway.
Male wistar rats were treated with (AII) or without (CTR) AII (1 mg/kg/d, 7d). AII-treated animals were further randomized to recieve either metoprolol (Meto, 100 mg/kg/d) or placebo. Endothelial function was studied by isometric tension studies using the vasorelaxant acetylcholine. Superoxide production was assessed by lucigenin chemiluminescence (LDCL) and dihydroethidium staining. NO was quantified using electron spin resonance spectroscopy. Activity of the NO downstream target cGK-I was studied by determining the phosphorylation status (pVASP/VASP) of the vasodilator stimulated protein (VASP) at serine 239. AII-infusion caused marked attenuation of the acetylcholine-response. Superoxide was increased in aortas from AII-treated animals. NO-bioavailability and cGK-I activity were decreased. Metoprolol significantly improved the abnormalities caused by AII-infusion.
We conclude that in experimental hypertension metoprolol, in addition to its mere antihypertensive effects, positively modulates the level of oxidative stress that accounts for cardiovascular pathology.

	Acetylcholine (ED50)	LDCL	NO	pVASP/VASP
CTR	-7.55 (LogM)	84	100%	100%
CTR+Meto	-7.55 (LogM)	67	91%	114%
AII	-6.40 (LogM)	145	54%	37%
AII+Meto	-7.05 (LogM)	115	92%	92%